

<110> Korea Advanced Institute of Science and Technology

<120> CONSTRUCTION OF NOVEL STRAINS CONTAINING MINIMIZING  
GENOME BY Tn5-COUPLED Cre/loxP EXCISION SYSTEM

<130> 02730.0020.PCUS00

<140> 10/505,328

<150> PCT/KR02/02033

<151> 2002-10-31

<150> KR 10-2002-0009647

<151> 2002-02-22

<160> 13

<170> KopatentIn 1.71

<210> 1

<211> 2437

<212> DNA

<213> Artificial Sequence

<220>

<223> TnKGloxP

<400> 1  
attcaggctg cgcaactgtt gggaagggcg atcgggtgagg gcctcttcgc tattacgccca 60  
gctgtctctt atacacatct caaccatcat cgatgaattc gagctcggta ccggggttga 120  
actgcggatc ttgcggccgc aaaaattaaa aatgaagttt tgacgggtatc gaacccacaga 180  
gtccccgtca gaagaactcg tcaagaaggc gatagaaggc gatgcgctgc gaatcgggag 240  
cggcgatacc gtaaagcacg aggaagcggc cagcccatc gccccaagc tcttcagcaa 300  
tatcacgggt agccaacgct atgtcctgat agcgggtccgc cacaccacgc cgccacagct 360  
cgatgaatcc agaaaaagcg ccattttcca ccatgatatt cggaagcagc gcacgcgcatt 420  
gggtcacgac gagatcctcg ccgtcgggca tccgcgcctt gagcctggcg aacagttcgg 480  
ctggcgcgag cccctgatgc tcttcgtcca gatcaccctg atcgacaaga ccggcttcca 540  
tccgagtagc tgctcgtcgc atcgcatgtt tcgcttggtg gtcgaatggc caggtagccg 600  
gatcaagcgt atgcagccgc cgcattgcat cagccatgat ggatacttct tcggcaggag 660  
caagggtgaga tgacaggaga tctgccccg gcacttcgcc caatagcagc cagtcccttc 720  
ccgcttcagt gacaacgtcg agcacagctg cgcaaggaa ccccgctcgt gccagccacg 780  
atagccgcgc tgcctcgtct tggagttcat tcagggcacc ggacaggctg gtcttgacaa 840

aaagaaccgg gcgcccctgc gctgacagcc ggaacacggc ggcatcagag cagccgattg	900
tctgttgtgc ccagtcatag ccgaatagcc tctccaccca agcggccgga gaacctgcgt	960
gcaatccatc ttgttcaatc atgcgaaacg atcctcatcc tgtctcttga tccactagat	1020
tattgaagca tttatcaggg ttattgtctc atgagcggat acatatttga atgtatttag	1080
aaaaataaac aaataggggg tccgcgcaca ttccccgaa aagtgcacc tgcatcgatg	1140
aattgatccg aagttcctat tctctagaaa gtataggaa cttcgaattg cgacaagctt	1200
gatctggcct atcgaaatta atacgactca ctatagggag accggaattc attatttcta	1260
gagctcatcc atgccatgtg taatcccagc agcagttaca aactcaagaa ggaccatgtg	1320
gtcacgcctt tcgttgggat ctttcgaaag ggcagattgt gtcgacaggt aatggttgtc	1380
tggtaaaaag acagggccat cgccaattgg agtattttgt tgataattgt ctgctagtgtg	1440
aacggatcca tcttcaatgt tgtggcgaat ttgaagtta gctttgattc cattcttttg	1500
tttgtctgcc gtgatgtata catttgtgtg gttatagttg tactcgagtt tgtgtccgag	1560
aatgtttcca tcttctttta aatcaatacc ttttaactcg atacgattaa caagggtatc	1620
accttcaaac ttgacttcag cacgcgtctt gtagttcccg tcactcttga aagatatagt	1680
gcgttctcgt acataacctt cgggcattgg actcttgaaa aagtcattgc gtttcatatg	1740
atccggataa cgggaaaagc attgaacacc ataagagaaa gtagtgacaa gtgttggccca	1800
tggaacaggt agttttccag tagtgcaaat aaatttaagg gtaagtttcc cgtatgttgc	1860
atcaccttca cctctctcac tgacagaaaa tttgtgccca ttaacatcac catctaattc	1920
aacaagaatt gggacaactc cagtgaanaa ttcttctcct ttactcattt ttctaccgg	1980
taccggggga tctcttagag tcgacctgca ggcattgcaag cttggcgtaa tcatggctcat	2040
agctgtttcc tgttgaaat tgttatccgc tcacaattcc acacaacata cgagccggaa	2100
gcataaagtg taaagcctgg ggtgccta at gagtgagcta actcacatta attgcgttgc	2160
gctcactgcc cgctttccag tcgggaaatc caaggcgcaa ttcgagctcg gtaccgggcc	2220
ccccctcgag ggaccttaata acttcgtata gcatacatta tacgaagtta tattaagggg	2280
tccggatcct cttagagtaga cctctagagt cgacctgcag gcattgcaag tttagggttg	2340
agatgtgtat aagagacagc tgcattaatg aatcgcccaa cgcgcggggg gaggcggttt	2400
gcgtattggg cgctcttcgg cttctcgcct cactgac	2437

<211> 1511  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> TnCloxP

<400> 2  
 attcaggctg cgcaactgtt ggggaagggcg atcgggtgctg gcctcttcgc tattacgcc 60  
 gctgtctctt atacacatct caaccatcat cgatgaattc gagctcggta ccgcaaaaat 120  
 taaaaatgaa gttttaaatc aatctaaagt atatatgagt aaacttggtc tgacagttac 180  
 caatgcttaa tcagtggagc accaataact gccttaaaaa aattacgccc cgccttgcca 240  
 ctcatcgag tactgttgta attcattaag cattctgccg acatggaagc catcacagac 300  
 ggcatgatga acctgaatcg ccagcggcat cagcaccttg tcgccttgcc tataatattt 360  
 gccatgggtg aaaacggggg cgaagaagt gtccatattg gccacgttta aatcaaaact 420  
 ggtgaaactc acccagggat tggctgagac gaaaaacata ttctcaataa accctttagg 480  
 gaaataggcc aggttttcac cgtaacacgc cacatcttgc gaatatatgt gtgaaaactg 540  
 ccggaaatcg tcgtggtatt cactccagag cgatgaaac gtttcagttt gctcatggaa 600  
 aacggtgtaa caagggtgaa cactatccca tatcaccagc tcaccgtctt tcattgccat 660  
 acggaatttc ggatgagcat tcacagggcg ggcaagaatg tgaataaagg ccggataaaa 720  
 cttgtgctta tttttcttta cggctcttaa aaaggccgta atatccagct gaacggtctg 780  
 gttataggta cattgagcaa ctgactgaaa tgccctcaaaa tgttctttac gatgccattg 840  
 ggatatatca acggtggtat atccagtgat tttttcttc attttagctt ccttagcttc 900  
 tgaaaatctc gataactcaa aaaatacgcc cggtagtgat cttatttcat tatggtgaaa 960  
 gttggaacct cttacgtgcc gatcaacgtc tcattttcgc caaaagttag ccaggggctt 1020  
 cccggtatca acaggggacac caggatttat ttattctgct aagtgtatct ccgtcacagg 1080  
 tatttattcg gcgcaaatg cgtcgggtg tgctgccaac ttactgattt agtgatgat 1140  
 ggtgtttttg aggtgctcca gtggtctctg tttctatcag catcgatgaa ttgatccgaa 1200  
 gttctattc tctagaaagt ataggaaact cgaattgtcg acaagcttga tctggcttat 1260  
 cgaaattaat acgactcact ataggagac cggaattcga gctcgtgacc gggccccccc 1320  
 tcgagggacc taataacttc gtatagcata cattatacga agttatatta agatcctcta 1380  
 gagtgcacct cgaggcatgc aagcttcagg gttgagatgt gtataagaga cagctgcatt 1440

aatgaatcgg ccaacgcgcg gggagaggcg gtttgcgtat tgggcgtct tccgcttcct 1500  
cgctcactga c 1511

<210> 3  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Outer end transposon recognition (OE) sequence

<400> 3  
ctgtctctta tacacatct 19

<210> 4  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> loxP site

<400> 4  
ataacttcgt atagcataca ttatacgaag ttat 34

<210> 5  
<211> 996  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> KmR gene

<400> 5  
gcaaaaatta aaaatgaagt ttgacggta tcgaacccca gagtcccgt cagaagaact 60  
cgtaagaag gcgatagaag gcgatcgct gcgaatcggg agcggcgata ccgtaaagca 120  
cgaggaagcg gtcagcccat tcgcccga gctcttcagc aatatacagg gttagccaagc 180  
ctatgtctcg atagcgggtcc gccacacca gccggccaca gtcgatgaat ccagaaaagc 240  
ggccatttc caccatgata ttcggaagc aggcacgccc atgggtcagc acgagatcct 300  
cgccgctggg catccgcgcc ttgagcctgg cgaacagttc ggctggcgcg agcccctgat 360  
gctcttcgtc cagatcatcc tgatcgacaa gaccggcttc catccgagta cgtgctcgct 420  
cgatgcgatg ttctcgcttg tggtcgaatg ggcaggtagc cggatcaagc gtatgcagcc 480

gcgcattgc atcagccatg atggatactt tctcggcagg agcaaggga gatgacagga	540
gatcctgcc cggcacttcg cccaatagca gccagtcct tcccgcttca gtgacaacgt	600
cgagcacagc tgcgcaagga acgcccgtcg tggccagcca cगतagccgc gctgcctcgt	660
cttgagttc attcaggga cgggacaggt cggctctgac aaaaagaacc gggcgccct	720
gcgctgacag ccggaacacg gcggcatcag agcagccgat tgtctgtgt gccagtcac	780
agccgaatag cctctccacc caagcggccg gagaacctgc gtgcaatcca tcttgttcaa	840
tcatgcgaaa cgatcctcat cctgtctctt gatccactag attattgaag catttatcag	900
ggttattgtc tcatgagcgg atacatatctt gaatgtattt agaaaaataa acaaataggg	960
gttccgcgca catttccccg aaaagtgcc cctgca	996

<210> 6  
 <211> 947  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> GFP gene

<400> 6	
attatttga gagctcatcc atgccatgtg taatcccagc agcagttaca aactcaagaa	60
ggaccatgtg gtcacgcttt tcgttgggat ctttcgaaa ggagattgt gtgcagaggt	120
aatggttgtc tggtaaaagg acagggccat cgccaattgg agtattttgt tgataatggt	180
ctgctagtgt aacggatcca tcttcaatgt tgtggcgaat tttgaagtta gctttgattc	240
cattcttttg tttgtctgcc gtgatgtata cattgtgtga gttatagttg tactcgagtt	300
tgtgtccgag aatgtttcca tcttcttta aatcaatacc ttttaactcg atacgattaa	360
caagggtatc accttcaaac ttgacttcag cagcgtctt gtatgtccgc tcatctttga	420
aagatatagt cggttcctgt acataacctt cgggcatggc actcttgaaa aagtcatgcc	480
gtttcatatg atccggataa cgggaaaagc attgaacacc ataagagaaa gtagtgacaa	540
gtgttgacca tggaaacaggt agttttccag tagtgcaaat aaatttaagg gtaagttttc	600
cgtatgttgc atcaccttca cctctccac tgacagaaaa tttgtgccca ttaacatcac	660
catctaattc aacaagaatt gggacaactc cagtgaagg ttcttctcct ttactcattt	720
tttctaccgg taccgggga tctctagag tcgacctgca ggcagtcaag cttggcgtaa	780
tcatggatcat agctgtttcc tgtgtgaaat tgttatccgc tcacaattcc acacaacata	840

cgagccggaa gcataaagt taaagcctgg ggtgccta atgtgagcta actcacatta 900  
attgcgttgc gctcactgcc cgctttccag tcgggaaatc caagggc 947

<210> 7  
<211> 1069  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> CmR gene

<400> 7  
gcaaaaatta aaaatgaagt tttaaatcaa tctaaagtat atatgagtaa acttggctcg 60  
acagttacca atgcttaatc agtgaggcac caataactgc cttaaaaaaa ttacgccccg 120  
ccctgccact catcgagta ctgttgaat tcattaagca ttctgccgac atggaagcca 180  
tcacagacgg catgatgaac ctgaatcgcc agcggcatca gcacctgtc gccttgcgta 240  
taatatattgc ccatgggtgaa aacgggggag aagaagtgt ccatattggc cacgtttaaa 300  
tcaaaactgg tgaaaactcac ccagggattg gctgagacga aaaacatatt ctcaataaac 360  
cctttagga aataggccag gttttaccg taacacgcca catcttgcga atatattgtt 420  
agaaaactgc ggaaatcgtc gtggtattca ctccagagcg atgaaaacgt ttcagtttgc 480  
tcattgaaaa cggtgttaaca aggtgaaca ctatcccata tcaccagctc accgtctttc 540  
attgccatac ggaatttcgg atgagcattc atcaggcggg caagaatgtg aataaaggcc 600  
ggataaaact tgtgcttatt tttctttacg gtctttaaaa aggccgta atccagctga 660  
acggtctggt tataggtaca ttgagcaact gactgaaatg cctcaaaatg ttctttacga 720  
tgccattggg atatatcaac ggtggtatat ccagtgtatt tttctccat tttagcttcc 780  
ttagctctcg aaaactctga taactcaaaa aatacggcgg gtatgtatct ttttccatta 840  
tggtgaaaat tggaacctct tacgtgccga tcaacgtctc attttcgcca aaagtggcc 900  
cagggtctcc cggtatcaac agggacacca ggatttattt attctcgcaa gtgatcttcc 960  
gtcacaggta tttattcgcc gcaaatgctg tcgggtgatg ctgccaaact actgatattag 1020  
tgtatgatgg tgtttttgag gtgctccagt ggcttctgtt tctatcagc 1069

<210> 8  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>  
 <223> primer-pMOD<MCS>FP-1

<400> 8  
 attcaggctg cgcaactgt 19

<210> 9  
 <211> 22  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> primer-pMOD<MCS>RP-1

<400> 9  
 tcagtgagcg aggaagcgga ag 22

<210> 10  
 <211> 28  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> primer-Tn5Ext

<400> 10  
 agcatacatt atacgaagtt atattaag 28

<210> 11  
 <211> 35  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> primer-Arb1

<220>  
 <221> misc\_feature  
 <222> (21)..(30)  
 <223> n is a or g or c or t

<400> 11  
 ttgagcgata gacgtacgat nnnnnnnnnn gatat 35

<210> 12  
 <211> 20  
 <212> DNA  
 <213> Artificial Sequence

<220>

<223> primer-Arb2

<400> 12

ttgagcgata gacgtacgat

20

<210> 13

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> primer-Tn5Int

<400> 13

tcgacctgca ggcattgcaag cttca

25